IN THE SPECIFICATION

Page 4, replace paragraph **[0009]** with the following: --

[0009] Two rifampicin-resistant attenuated *F. columnare* ~~isolate~~ isolates, were deposited on June 20, 2000 under the provisions of the Budapest Treaty in the Agricultural Research Service Culture Collection in Peoria, IL, and have been assigned Deposit ~~No.'s~~ Numbers B-30303 and B-30304~~, respectively~~. --

Page 5, replace paragraph **[0011]** with the following: --

[0011] The starting material for use in preparing the vaccines of the invention is any attenuated *F. columnare* bacterium such as those reported ~~supra~~ *, supra*. Serial passage of the isolate of *F. columnare* over increasing concentrations of rifampicin produces strains with an attenuated pathogenicity efficacious for the preparation of live vaccines. The attenuation achieved by high-level serial passage in culture on increasing concentrations of rifampicin virtually eliminates the pathogenicity of the bacterium toward fish. The native strain of *F. columnare* should be passaged a sufficient number of times such that in its new attenuated form it no longer possesses the ability of causing the disease state known as columnaris in catfish. The methodology for attenuation by serial passage is well known and documented in the art as exemplified by Schurig et al. [*Vet. Micro.* 28, 171-188

(1991)], hereby incorporated by reference, who created vaccines based on modified live rifampicin-resistant *Brucella* species. --

Pages 6 and 7, replace paragraph **[0013]** with the following: --

[0013] A positive vaccinal effect is indicated by a RPS equal to or greater than 60%. Typically, vaccination is carried out by exposing fish by immersion in water containing about 1 x 10^6 CFU/ml of attenuated *Flavobacterium columnare* for 15 minutes at a density of about 50 fish/L and a temperature of about 25°C. CFU denotes colony forming units of *F. columnare*. These parameters may be varied as desired such that a sufficient level of vaccination is acquired without induction of stressful conditions or loss of fish. Useable concentrations of *Flavobacterium columnare* are considered to range from about 5 x 10^5 to about 1 x 10^8 CFU/ml of immersion medium. Useable vaccination times are seen to range from about 1 minute to about 60 minutes, preferably from about 2 minutes to about 15 minutes. Temperature of the inoculation media may range within the physiologically acceptable limits of the fish involved, for channel catfish preferably from about 18°C to about 28°C, most preferably from about 22°C to about 26°C. Concentrations of fish treated in the inoculation medium typically range from about 50 to about 100 fish/L, but, in the alternative, may be determined on a weight

basis and range from about 0.5 to about 2.5 kg/L. The vaccine can be effectively administered ~~anytime~~ any time after the fish attains immunocompetence, which for channel catfish is at about ~~the second day~~ two to fourteen days post-hatch. Other species of fish susceptible to *F. columnare* can be immunized after 21-30 days post-hatch or when they become immunocompetent to modified live vaccine administered by immersion. --

Pages 8 and 9, replace paragraph **[0017]** with the following: --

[0017] Modified *Cytophaga* agar plates for the cultivation of *Flavobacterium columnare* were made according to the procedure of Klesius et al. (Effect of feed deprivation on innate resistance and antibody response to *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus*. Bulletin European Association of Fish Pathologists, 19(4), 156-158, 1999). 1.0 g tryptone, 0.5 g yeast extract, 0.2 g beef extract, 0.2 g sodium acetate and 9.0 g of agar were added to one liter of distilled water. The ~~media and agar were~~ medium was heated until dissolution. The ~~media~~ medium was then autoclaved at 121-124°C for 15 minutes, ~~the media was then~~ poured into sterile petri dishes (15 ml per dish) and allowed to solidify before refrigeration. --

Pages 9 and 10, replace paragraph **[0018]** with the following:

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[0018] Native isolates of *Flavobacterium columnare* were obtained from sick catfish or previously obtained lyophilized stocks. Isolates of *F. columnare* were then identified by standard biochemical tests as set forth in Bergey's Manual of Determinative Bacteriology prior to use in rifampicin resistant *F. columnare*. After identification, the process of forming rifampicin resistant isolates of *F. columnare* was begun. Rifampicin supplemented modified *Cytophaga* agar plates were prepared as follows: Modified *Cytophaga* agar was made as described above and sterilized at 121-124°C for 15 minutes. After sterilization, the correct amount of rifampicin was added to the media prior to its solidification and 15 ml of the resulting mixture was poured into separate petri dishes and allowed to solidify prior to refrigerated storage. Initial cultures of the native isolates of *F. columnare* were grown on modified *Cytophaga* agar plates which were incubated at 20-25°C for 24-48 hours or until 1-2 mm yellow, rhizoid colonies were observed. A single *F. columnare* colony was then picked with a sterile inoculating loop and streaked onto a rifampicin supplemented modified *Cytophaga* agar plate containing the correct concentration of the antibiotic. For the initial passage, rifampicin was present in the modified *Cytophaga* agar at a

concentration of 5 μg/ml. The ~~rifampicin supplemented~~ rifampicin-supplemented modified *Cytophaga* agar which was streaked with the aforementioned native isolate of *F. columnare* was then incubated for 24-48 hours at 20-25°C and observed for bacterial growth. Single colonies of *F. columnare* which grew on the ~~rifampicin supplemented media~~ rifampicin-supplemented medium were then picked and placed onto the next concentration of rifampicin (10 μg/ml) modified *Cytophaga* agar plates. If growth occurred, a single colony was harvested and placed on an agar ~~media~~ medium containing the next higher concentration of rifampicin (20 μg/ml). If the harvested colony failed to grow, it was repeatedly passed on a ~~media~~ medium containing the last concentration of rifampicin at which growth successfully occurred, before being placed on the next higher concentration of ~~rifampicin containing media~~ rifampicin-containing medium. This process was repeated until a colony capable of growing on a ~~media~~ medium containing a rifampicin concentration of 200 μg/ml was created. --

Pages 10 and 11, replace paragraph **[0019]** with the following: --

[0019] *Flavobacterium columnare* isolate ARS-1 was passaged on increasing concentrations of rifampicin (Sigma Chemical Company, St. Louis, MO) supplemented modified *Cytophaga* agar to a final

concentration of 200 µg/ml rifampicin for 243 passages. The resultant mutants (i.e., two colonies from the original passage that grew and were passaged), designated B-30303 and B-30304, are differentiated from the parent microorganism because they can survive and reproduce on a ~~media~~ medium containing 200 µg/ml rifampicin without negative effect. Biochemical characteristics of the *F. columnare* B-30303 and B-30304 are identical to *F. columnare* as described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), herein incorporated by reference. --

Page 15, replace Table 3 with the following: --

Table 3

PROTECTION AGAINST COLUMNARIS DISEASE AFTER IMMERSION VACCINATION OF CHANNEL CATFISH WITH *FLAVOBACTERIUM COLUMNARE* B-30303 VACCINE

Group	Treatment	No. Dead/ No. Total	Mean % Mortality (SEM[1])	Relative Percent Survival[2]
A	Vaccinated 1×10^6 CFU/ml	2/150	1.33 (0.67)[a]	96.4
B	Vaccinated 5×10^6 CFU/ml	7/150	4.67 (0.67)[a]	~~97.3~~ 87.3
C	Control	55/150	36.67 (17.7)[b]	---
D	Control Non-challenged	3/150	2.00 (1.15)[a]	---

[1]Means with different superscripts are significantly different at $p<0.05$ using Duncan's Multiple range test for differences (SAS Inc., 1997).
[2]Relative percent survival calculated according to Amend (1981).
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Pages 16, replace paragraph **[0025]** with the following: --

[0025] Culture was also attempted after the isolates recovered from cold storage had grown on the non-rifampicin supplemented plates for 48 hours. Single colonies of the recovered bacteria that were growing on *Cytophaga* agar were picked and streaked onto *Cytophaga* agar supplemented with 5 μg rifampicin/ml. No growth occurred on *Cytophaga* agar supplemented with 5 μg rifampicin/ml ~~following the use of 48 hours cultures of recovered *F. columnare*.~~

~~Lack of growth on *Cytophaga* agar supplemented with 5 μg rifampicin/ml demonstrated~~ demonstrating that these isolates were not viable candidates for development of modified live *F. columnare* vaccines because rifampicin resistance could not be induced in these native *F. columnare* isolates (see Table 4). ~~Failure of induction of rifampicin resistance was demonstrated by no growth on rifampicin supplemented *Cytophaga* agar. This testing shows~~ These results support the unexpected nature of the success achieved in Example 1. --